

Supplementary Figure 3

Immunohistochemical assessment of photo-induced damage.

Representative images of brain sections containing the laser scanned regions that were immunostained with antibodies to detect microglial (anti-lba1; green) and astrocyte (anti-GFAP; red) activation. All brains (with the exception of control) were illuminated for continuous 20min under the condition stated below. Arrows indicate the approximate center of imaging site. (a) Immunolabeled sections of a mouse brain imaged with 2D s-TeFo scanning at 350µm depth with 220 mW effective average laser power. Condition is similar to Fig. 2e. (b) Same as in a, but with 3D s-Tefo from 0-500µm depth and 70-200mW cycled averaged laser power. Condition is the same as in Fig. 4. (c) Control group that underwent window surgery but was not exposed to laser illumination. (d) Positive control group that was subject to 2D s-TeFo at 200µm depth and high laser power (400mW). (e,f) Intensity of immunolabeling, as fraction compared to control area, for mice illuminated with different laser intensities and scanning modalities, as indicated. 2P – standard two-photon microscopy.